Immune and non-immune mediated roles of regulatory T-cells during wound healing

Immune and non-immune mediated roles of regulatory T-cells during wound healing

Dietmar M. Zaiss[1], Carlos M. Minutti[1,2] and Johanna A. Knipper[1]

[1] Institute of Immunology and Infection Research, University of Edinburgh, Charlotte Auerbach Road, Edinburgh EH9 3FL, UK.
[2] Immunobiology Laboratory, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK.

Correspondence should be addressed to: Dietmar.Zaiss@ed.ac.uk

Abstract:

The immune system has a well-established contribution to tissue homeostasis and wound healing. However, in many cases immune responses themselves can cause severe tissue damage. Thus, the question arose to which extent cells of the immune system directly contribute to the process of wound healing and to which extent the resolution of excessive immune responses may indirectly contribute to wound healing. FoxP3 expressing CD4 T-cells, so-called regulatory T-cells (T_{regs}), have an important contribution in the regulation of immune responses; and, in recent years, it has been suggested that T_{regs} next to an immune regulatory, “damage-limiting” function may also have an immune independent, “damage-resolving” direct role in wound healing. In particular, the release of the EGF-like growth factor Amphiregulin by tissue-resident T_{regs} during wound repair suggested such a function. Our recent findings have now revealed that Amphiregulin induces the local release of bio-active TGFβ, a cytokine involved both in immune regulation as well as in the process of wound repair. In light of these findings, we discuss whether, by locally activating TGFβ, T_{reg}-derived Amphiregulin may contribute to both wound repair and immune suppression. Furthermore, we propose that T_{reg}-derived Amphiregulin in an autocrine way may enable an IL-33 mediated survival and expansion of tissue-resident T_{regs} upon injury. Furthermore, T_{reg}-derived Amphiregulin may contribute to a constitutive, low-level release of bio-active TGFβ within tissues, leading to continuous tissue regeneration and to an immune suppressive environment, which may keep inflammation prone tissues in an homeostatic state.

Regulatory T-cells have an established role in wound healing:

Following a breach of tissue homeostasis (be it by infection or tissue injury) a highly complex, but also well-orchestrated process of local inflammation occurs which guides local wound healing [1, 2]. This process can roughly be separated into three distinct, consecutive but overlapping phases. These phases are traditionally divided into a pro-inflammatory initiation phase, a tissue formation phase and a resolution and tissue re-organisation phase [1]. During each of these phases different types of leukocytes infiltrate the site of tissue damage and play distinct, clearly defined roles [1]. The injury itself induces a pro-inflammatory initiation phase, during which neutrophils and pro-inflammatory monocytes are recruited to the site of injury [2]. These cells contribute to host defence and the removal of cell debris and of necrotic cells. The pro-inflammatory initiation phase then transitions into the so-called tissue formation phase, in which inflammation is already dampened and a differentiation of infiltrating monocytes into alternatively activated macrophages occurs. During this phase, angiogenesis and cell proliferation lead to a closure of the wound. In the following, the so-called resolution or tissue-remodelling phase, the immune response is actively suppressed and excessive tissue and cellular matrix growth is reversed [2]. This process of wound healing has to be well orchestrated and disruption of this process, for instance due to the infection of the healing wound, can lead to a failure of wound healing or excessive scar formation [3].

The transition from the pro-inflammatory initiation phase into the tissue formation phase and the resolution phase is critically controlled by immune regulatory mechanisms. In particular FoxP3-expressing regulatory CD4 T-cells (T_{regs}) appear to play a critical role in this transition. T_{regs} rapidly migrate to and accumulate at sites of inflammation, such as at sites of injury [4-6]. Depletion of T_{regs} during the different phases of wound healing, for instance by the application of Diphtheria Toxin (DT) to FoxP3:DTR transgenic mice or by the injection of CD25-depleting antibodies, consistently led to aggravated inflammation and deteriorated clinical outcomes in a number of different injury model systems in mice [4, 6-12].

For instance, in mouse models of myocardic infarction, the depletion of T_{regs} resulted in aggravated cardiac inflammation and deteriorated clinical outcome [9, 10]. In these experiments, T_{reg}-cell depletion was associated with increased neutrophil and monocyte infiltration and diminished alternatively-activated macrophage polarization [9]. In contrast, expansion of the T_{reg} compartment, for instance, via activation using super-agonistic anti-CD28 monoclonal antibody administration [9] or adoptive T_{reg} transfer [10], reduced infarct size and improved tissue remodelling and functional performance of the heart. Similarly, in mouse models of ischaemic-reperfused kidneys in *Rag1*$^{-/-}$ mice, the transfer of T_{regs} reduced the influx of neutrophils and macrophages, and diminished innate cytokine transcription in the kidney, resulting in diminished renal injury [7]. Also in mouse models of muscle injury, such as in the mdx mouse model of Duchenne muscular dystrophy [11] or acute muscle injury

[4], DTR-mediated depletion of T_{regs} in FoxP3:DTR transgenic mice exacerbated muscle injury and the severity of muscle inflammation, which was associated with a prolonged infiltration of pro-inflammatory monocytes and a bias towards classically-activated macrophages. In contrast, expansion of T_{regs} via the application of IL-2/anti-IL-2 complexes in mdx mice led to decreased myofiber injury and suppressed inflammation in muscles associated with muscle fiber injury [11].

Thus, taken together, these findings clearly support the conclusion that T_{regs} critically contribute to the process of wound healing

Immune-mediated roles of regulatory T-cells during wound healing:

One of the underlying mechanisms associated with T_{reg}-mediated wound healing is assumed to be the suppression of pro-inflammatory stimuli. Such T_{reg}-mediated immune suppression appears to be a critical factor allowing for the progression of the wound healing process [13]. T_{regs} utilize a number of different mechanisms to locally mediate their immune suppressive function [14]. These mechanisms include the local activation of bio-active TGFβ [15, 16], the secretion of the immune suppressive cytokine IL-10 [17] or the conversion of local adenosine monophosphate into adenosine [18]. Accordingly, in mdx mice treated with IL-2/anti-IL-2 complexes more T_{regs} and increased IL-10 concentrations were found in injured muscles [11]. Also, in an ischaemic-reperfused kidney model and an experimental brain ischemia model in *Rag1*$^{-/-}$ mice, the adoptive transfer of wild-type, but not of IL-10-deficient T_{regs}, was sufficient to ameliorate tissue injury [7, 8]. Furthermore, in a model of LPS-induced acute lung injury (ALI), the transfer of wild-type, but not of CD73-deficient Tregs ameliorated lung injury in *Rag*$^{-/-}$ mice; strongly suggesting that the CD73-mediated adenosine generation and thus induced immune suppression by T_{regs} contributed to the restoration of tissue homeostasis [19].

Thus, the resolution of local inflammation is clearly a key function of T_{regs} during wound healing (Figure 1).

Non-immune mediated roles of regulatory T-cells during wound healing:

In recent years, it has further been suggested that T_{regs} may also directly contribute to wound healing, independent of their immune regulatory function [4, 20]. Such a notion was supported by the discovery that in several different tissues, such as the muscle [4], a specific subtype of T_{regs}, so called tissue-resident T_{regs} [21, 22], expresses the EGF-like growth factor Amphiregulin.

Amphiregulin is an EGF-like growth factor associated with a number of different physiological processes, such as tissue homeostasis, inflammation and immunity [23, 24]. With regard to tissue homeostasis, a direct role for Amphiregulin has for instance recently been demonstrated for the gingiva [25]. The gingiva is a key oral barrier site and Amphiregulin gene-

deficient mice showed at steady state a substantially elevated level of oral, periodontal pathology in comparison to wild-type mice [25]. Furthermore, during wound healing, a direct role of Amphiregulin has been suggested. For instance during infections, the injection of recombinant Amphiregulin (rAREG) has in several different experimental settings demonstrated the amelioration of symptoms, such as during Influenza infection [26, 27] or following viral-bacterial co-infections [28]. In line with these findings, also during wound healing following muscle injury, the injection of rAREG enhanced the restoration of injured muscles and the presence of rAREG enhanced the differentiation of muscle stem cells *in vitro*. These findings were further corroborated by findings from the Rudensky group, using a mouse strain with a T_{reg}-specific deficiency of Amphiregulin (*FoxP3:cre x Areg$^{fl/fl}$*) [20]. These mice showed a substantially more severe form of symptoms following Influenza infection than wild-type mice [20], suggesting a direct role of T_{reg}-derived Amphiregulin in wound repair.

Other studies further supported the finding that during wound healing T_{regs} may have, independent of immune modulatory function, a complementary, regenerative role. For instance, in a model of lysolecithin-mediated demyelination in the spinal cord of mice, T_{regs} were found to directly promote oligodendrocyte differentiation and myelin production [29]. Depletion of T_{regs} led to a substantially impaired remyelination and oligodendrocyte differentiation, which could be reversed by the adoptive transfer of T_{regs}. *In vitro* studies then suggested that T_{reg}-derived matricellular protein CCN3 - a protein known to induce the expression of TGFβ-related BMP proteins [30] - directly promoted oligodendrocyte progenitor cell differentiation and myelination [29]. These data suggest that also *in vivo* T_{reg}-derived CCN3 may play a similar, wound healing supportive role in injured spinal cord tissue.

In addition, in a study using established models of tissue regeneration in zebrafish, the conditional ablation of FoxP3 expressing zebrafish T_{regs} hampered organ regeneration [31]. Dependent on the injured organ, infiltrating T_{regs} stimulated the proliferation of tissue precursor cells through the secretion of organ-specific regenerative factors, such as Ntf3 for the spinal cord, Nrg1 for the heart and Igf1 for the retina. Moreover, when Foxp3-deficient zebrafish T_{regs} infiltrated the injured organs, they failed to express regenerative factors and thus could not contribute to wound healing [31].

Combined, these findings strongly suggest that T_{regs}, in addition to an immune modulatory, “damage-limiting” function, may also have a direct wound repair, “damage-resolving” function (Figure 1).

The role of Amphiregulin in immune regulation and wound healing:

The concept of such a double function of T_{regs} for “damage-limiting” and “damage-resolving” during wound healing is rather appealing. Nevertheless, a number of different findings suggest that, on a molecular level, the two functions might not easily be separable.

In mammalians the concept of a distinction of these two functions is mainly based on the finding that several types of tissue-resident T_{regs} express Amphiregulin and that the injection of rAREG supported the process of wound healing in several different model systems. However, the exact mechanism by which Amphiregulin contributes to wound healing had remained unresolved, while at the same time Amphiregulin had been shown before to enhance the suppressive capacity of T_{regs} *in vitro* and *in vivo* [32-36].

We recently found that one critical function of Amphiregulin is to locally activate latent TGFβ [37]. Thus, via this local release of TGFβ, Amphiregulin may contribute to both the local suppression of inflammation as well as to the local differentiation of tissue stem cells and in this way to the process of wound healing and restoration of tissue homeostasis. In the following, we will discuss, how this novel insight may influence our understanding of T_{reg}-derived Amphiregulin for tissue homeostasis, wound repair and immune suppression.

It has been well established that the receptor of Amphiregulin, the Epidermal Growth Factor Receptor (EGFR), contributes to wound healing [38]. So far, it has mainly been assumed that this receptor mediates its function by inducing the proliferation of epithelial cells within the wound [38]; and, in line with such an assumption, it has recent indeed been published from the group of Belkaid that a specific function for CD8 T-cell derived Amphiregulin is the induction of keratinocyte expansion within a healing skin wound [39]. However, for many other epithelial and mesenchymal cell types, the low-affinity ligand of the EGFR Amphiregulin is actually not a particular good mitogen. In contrast to the high-affinity ligands of EGFR, such as EGF, TGFα or HB-EGF, which activate the proliferation-stimulating MAPK signalling pathway [40, 41], Amphiregulin preferentially induces the phosphorylation of EGFR-Y992 and thus the activation of PLCγ signalling pathway [42, 43]. Thus, it is highly unlikely that Amphiregulin substantially contributes to wound healing by inducing the proliferation of epithelial cells at the site of wounding; and, thus, the underlying mechanism by which Amphiregulin contributes to wound healing has largely remained unresolved.

We recently discovered that Amphiregulin induced the local activation of TGFβ [37]. In mouse models of CCl_4-induced acute liver damage and of *Nippostrongylus* infection-induced lung damage, we demonstrated that during wound healing Amphiregulin induced the TGFβ-mediated differentiation of tissue stem cells and in this way critically contributed to the restoration of tissue homeostasis (Figure 2). TGFβ is secreted in a latent form and only by releasing it from this latent complex becomes the bio-active form of TGFβ exposed [44]. One way of releasing bio-active TGFβ, is the activation of integrin-α_V containing complexes [45]. We found that Amphiregulin induced the activation of integrin-α_V containing complexes and thus the local activation of TGFβ. This local activation of TGFβ induced the differentiation of blood vessel-associated mesenchymal precursor cells, so called pericytes, into collagen-

producing myo-fibroblasts; a process, which critically contributed to the restoration of injured blood vessels and thus wound healing (Figure 2).

A number of growth factors and chemokine receptors as well as the TCR use such an "inside-out" activation of integrin complexes to establish immunological synapsis between T-cells and antigen-presenting cells [46] or to allow leukocytes to attach themselves to blood vessels in order to cross into inflamed tissues [47]. A critical step in this "inside-out" activation of integrin complexes is the sustained activation of the PLCγ signalling pathway [48]. Amphiregulin preferentially induces the PLCγ signalling pathway [42, 43] and is thus well situated to induce the activation of integrin-α_V containing complexes on target cells, which then results in the release of bio-active TGFβ from its latent form (Figure 3). Thus, taken together, our data show that one major function of Amphiregulin is the local induction of TGFβ.

Similar to Amphiregulin, also TGFβ has a double function during wound healing. For one, TGFβ induces the differentiation of tissue resident precursor cells; and, for the other, TGFβ is a key mediator of T_{reg}-mediated immune suppression [15]. To achieve TGFβ-mediated immune suppression, integrin-α_V containing complexes on T_{regs} have to be activated, which then release bio-active TGFβ [16]. A number of different groups have shown in *in vitro* suppression assays that rAREG enhances the suppressive capacity of T_{regs} [32, 34-36]. Also in *in vivo* settings Amphiregulin enhances the suppressive capacity of T_{regs} [32, 33]. For instance, in a T-cell transfer based colitis model, the titrated transfer of wild-type T_{regs} into *Rag1*$^{-/-}$ mice that had received naïve CD4 T-cells could in a dose-dependent manner suppress the induction of colitis, if the recipient *Rag1*$^{-/-}$ mice were on a wild-type background but not if the recipient *Rag1*$^{-/-}$ mice had been backcrossed onto an Amphiregulin-deficient background [32]. Furthermore, following the adoptive transfer of antigen-specific T_{regs}, these cells could suppress hapten-induced ear swelling in wild-type, but not in Amphiregulin gene-deficient mice. These findings clearly demonstrated that endogenous expression of Amphiregulin is essential to ensure efficient T_{reg} function. In line with this immune-suppressive function of Amphiregulin, it has been reported that also following ischaemic stroke T_{reg}-derived Amphiregulin contributes to neurological recovery by suppressing IL-6 expression within the brain and thus avoiding neurotoxic astrogliosis [6].

Although we so far have not yet formally addressed whether Amphiregulin also activates integrin-α_V containing complexes on T_{regs}, our pericyte-based finding of Amphiregulin-mediated TGFβ activation suggests that such a mechanism might also be the underlying effect by which Amphiregulin enhances T_{reg} function [32]. Nevertheless, in consequence, this TGFβ activating role of Amphiregulin also means that in those experimental settings, in which rAREG has been injected into mice, the injected rAREG may have induced the

release of bio-active TGFβ. This release of bio-active TGFβ may have enhanced the differentiation of tissue precursor cells, such as pericytes [37] or muscle satellite cells [4], and thus may have directly contributed to wound healing; or, at the same time, may have enhanced the suppressive capacity of T_{regs} and thus may also indirectly have contributed to wound healing. Thus, in two ways may have contributed to tissue repair (Figure 1).

The function of regulatory T-cell derived Amphiregulin:

In a similar way, it is possible that T_{reg}-derived Amphiregulin could induce the local release of bio-active TGFβ and in this way the differentiation of tissue precursor cells; thus, may directly be contributing to tissue repair. Nevertheless, while T_{regs} have to be considered the most prominent cell type mediating local immune regulation, T_{regs} are not the only Amphiregulin-producing cells within inflamed tissues; but, a wide range of other prominent Amphiregulin-producing cell types, such as eosinophils, have been shown before to also critically contributing to the process of wound healing [49].

Such a redundancy of cell types, which all could be potentially physiological relevant sources of Amphiregulin within injured tissues, raises the question what specific function T_{reg}-derived Amphiregulin might have. Amphiregulin expressing T_{regs} are typically restricted to tissue resident T_{reg} populations and thus suggest an organ-specific role for T_{reg}-expressed Amphiregulin [21]. These tissue resident T_{reg} populations also preferentially express the IL-33 receptor, T1/ST2 [6, 50, 51]. The role of T1/ST2 expression on T_{regs} currently remains unresolved. However, IL-33 is an important alarmin, released by dying cells and recent data strongly suggest that IL-33 signalling in T_{regs} provides a critical signal for T_{reg} accumulation and maintenance in inflamed tissues [6, 50, 52, 53]. However, also how IL-33 may contribute to such an expansion of T_{reg} populations at the site of inflammation remains largely unknown.

We have shown before that the EGFR forms hetero-complexes with T1/ST2 on Th2 cells [43]. These hetero-complexes allowed Th2 cells to efficiently activate the MAPK signalling pathway and thus to induce the expression of IL-13 upon exposure to IL-33 [43] (Figure 4). In CD4 T-cells, it has been shown that EGFR is one of the most prominently up-regulated trans-membrane receptors upon STAT5 activation [54]. We showed that both in Th2 cells as well as in T_{regs} the EGFR is strongly up-regulated upon activation [32, 43]. Also kinome profiling in human T_{regs} revealed that the EGFR is one of the strongest up-regulated kinases upon T_{reg} activation [55]. In line with these findings, the group of Rosenblum found induced EGFR expression in T_{regs} in a model of skin wounding in mice [12] and lineage-specific deletion of EGFR in T_{regs} resulted in reduced T_{reg} accumulation within the inflamed skin [12]. Thus, in this respect the phenotype of EGFR- and T1/ST2-deficient T_{regs} resembles each other, with both depicting a deficiency to expand at the site of inflammation.

These findings provide a scenario in which similar to Th2 cells also on T_{regs} T1/ST2 and the EGFR could form hetero-complexes that may allow for IL-33 induced activation of the MAPK signalling pathway (Figure 4). The MAPK signalling pathway is a pivotal signalling pathway for the transduction of mitogenic stimuli [41, 56]. Thus, such an IL-33 mediated activation of the MAPK signalling pathway might therefore be a possible mechanism that may explain for the observed expansion of tissue resident T_{reg} populations upon injection of rIL-33 [50, 52].

Such hetero-complexes between the EGFR and T1/ST2 on tissue resident T_{regs} could further enable these cells to become activated in a TCR independent way, as we have demonstrated before for Th2 cells [43]. Upon tissue damage, antigen-specific restimulation of tissue resident T_{reg} populations via MHC-II antigen presentation and TCR-mediated activation might not always be possible. Thus, the recognition of released IL-33 from damaged tissues may constitute an alternative pathway of activation for tissue-resident T_{reg} populations. Such an assumption is further supported by a study using a NOD based model of auto-immune diabetes. This study demonstrated that in specific T_{reg} populations with a low-affinity TCR for their cognate antigen preferentially expressed Amphiregulin, preferentially localized to the site of inflammation and functioned there in an antigen-independent way [57]. Thus, taken together, the combined expression of T1/ST2 and EGFR may enable tissue-resident T_{reg} populations to function in a MHC-II independent way.

In Th2 cells, the induced expression of Amphiregulin, in an autocrine way, enabled the formation of such hetero-complexes between EGFR and T1/ST2, and only upon expression of Amphiregulin could activated Th2 cells function in a MHC-II independent way [43]. Assuming a similar function for Amphiregulin in tissue resident T_{reg} populations, then the constitutive expression of Amphiregulin may keep these T_{regs} in a “poised” state (Figure 4). In such a “poised” state tissue resident T_{reg} populations would be able to rapidly respond to tissue damage and the exposure to IL-33.

Such a poised state of tissue resident T_{reg} populations could be critical during tissue injury, for instance due to the fact that IL-33 release from necrotic cells is a very early event during tissue injury in many tissues. Thus, this poised state may allow to already inducing the activation and expansion of T_{reg} populations at the site of injury, while the infiltration of T_{reg} populations derived from secondary lymphoid organs into injured tissues may so far not have been initiated yet [5, 6]. Thus, the constitutive expression of Amphiregulin by tissue-resident T_{regs} may constitute a first line of defence during wounding. However, also under steady state conditions, such a “poised” state of tissue resident T_{reg} populations could be contributing to tissue homeostasis; for instance in fatty tissues, in which a constant, IL-33 dependent but most likely antigen-independent activation of T_{reg} populations contributes to the control of local and systemic inflammation and metabolism.

Summary and out-look:

Taken together, a wide range of different publications in recent years strongly suggested that T_{regs} play an important role in wound healing, both by suppressing local inflammation and also by directly contributing to the wound healing process. The exact mechanism of how T_{regs} contribute to these processes has remained to be resolved and further research is necessary to fully resolve the specific function of T_{reg}-derived Amphiregulin. At this stage, it remains speculative to which extent T_{reg}-derived Amphiregulin main function might be to directly contribute to wound healing, and to which extent it contributes to the local release of bioactive TGFβ, which then has both a wound healing and immune suppressive function at the site of injury.

However, alternatively, T_{reg}-derived Amphiregulin may also contribute to the survival and expansion of tissue-resident T_{regs} upon injury. Since it was also further shown that T1/ST2 expressing T_{reg} populations preferentially express high levels of integrin-α_V and low levels of IL-10 [51], it is tempting to speculate that this constitutive expression of Amphiregulin by tissue-resident T_{regs} may also lead to a constitutive, low level release of bio-active TGFβ, which may contribute to on-going regeneration of tissues and to a low level immune suppressive environment in the surrounding of Amphiregulin-expressing tissue resident T_{reg} populations, which might be an evolutionary advantage for instance in inflammation prone tissues, such as fatty tissues.

References:

1. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. Science 2017; 356:1026-30.
2. Minutti CM, Knipper JA, Allen JE, Zaiss DM. Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol 2017; 61:3-11.
3. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 2005; 15:599-607.
4. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, et al. A special population of regulatory T cells potentiates muscle repair. Cell 2013; 155:1282-95.
5. Luo CT, Liao W, Dadi S, Toure A, Li MO. Graded Foxo1 activity in Treg cells differentiates tumour immunity from spontaneous autoimmunity. Nature 2016; 529:532-6.
6. Ito M, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. Nature 2019.
7. Kinsey GR, Sharma R, Huang L, Li L, Vergis AL, Ye H, et al. Regulatory T cells suppress innate immunity in kidney ischemia-reperfusion injury. J Am Soc Nephrol 2009; 20:1744-53.
8. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. Nat Med 2009; 15:192-9.
9. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, et al. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. Circ Res 2014; 115:55-67.
10. Sharir R, Semo J, Shimoni S, Ben-Mordechai T, Landa-Rouben N, Maysel-Auslender S, et al. Experimental myocardial infarction induces altered regulatory T cell hemostasis, and adoptive transfer attenuates subsequent remodeling. PLoS One 2014; 9:e113653.
11. Villalta SA, Rosenthal W, Martinez L, Kaur A, Sparwasser T, Tidball JG, et al. Regulatory T cells suppress muscle inflammation and injury in muscular dystrophy. Sci Transl Med 2014; 6:258ra142.
12. Nosbaum A, Prevel N, Truong HA, Mehta P, Ettinger M, Scharschmidt TC, et al. Cutting Edge: Regulatory T Cells Facilitate Cutaneous Wound Healing. J Immunol 2016; 196:2010-4.
13. Li J, Tan J, Martino MM, Lui KO. Regulatory T-Cells: Potential Regulator of Tissue Repair and Regeneration. Front Immunol 2018; 9:585.
14. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 2012; 30:531-64.
15. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006; 24:99-146.
16. Worthington JJ, Kelly A, Smedley C, Bauche D, Campbell S, Marie JC, et al. Integrin alphavbeta8-Mediated TGF-beta Activation by Effector Regulatory T Cells Is Essential for Suppression of T-Cell-Mediated Inflammation. Immunity 2015; 42:903-15.
17. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 2008; 28:546-58.
18. Kobie JJ, Shah PR, Yang L, Rebhahn JA, Fowell DJ, Mosmann TR. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. J Immunol 2006; 177:6780-6.
19. Ehrentraut H, Clambey ET, McNamee EN, Brodsky KS, Ehrentraut SF, Poth JM, et al. CD73+ regulatory T cells contribute to adenosine-mediated resolution of acute lung injury. FASEB J 2013; 27:2207-19.
20. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, et al. A Distinct Function of Regulatory T Cells in Tissue Protection. Cell 2015; 162:1078-89.
21. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009; 15:930-9.

22. Panduro M, Benoist C, Mathis D. Tissue Tregs. Annu Rev Immunol 2016; 34:609-33.
23. Berasain C, Avila MA. Amphiregulin. Semin Cell Dev Biol 2014; 28:31-41.
24. Zaiss DM, Gause WC, Osborne LC, Artis D. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. Immunity 2015; 42:216-26.
25. Krishnan S, Prise IE, Wemyss K, Schenck LP, Bridgeman HM, McClure FA, et al. Amphiregulin-producing gammadelta T cells are vital for safeguarding oral barrier immune homeostasis. Proc Natl Acad Sci U S A 2018; 115:10738-43.
26. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nat Immunol 2011; 12:1045-54.
27. Guo XJ, Dash P, Crawford JC, Allen EK, Zamora AE, Boyd DF, et al. Lung gammadelta T Cells Mediate Protective Responses during Neonatal Influenza Infection that Are Associated with Type 2 Immunity. Immunity 2018; 49:531-44 e6.
28. Jamieson AM, Pasman L, Yu S, Gamradt P, Homer RJ, Decker T, et al. Role of tissue protection in lethal respiratory viral-bacterial coinfection. Science 2013; 340:1230-4.
29. Dombrowski Y, O'Hagan T, Dittmer M, Penalva R, Mayoral SR, Bankhead P, et al. Regulatory T cells promote myelin regeneration in the central nervous system. Nat Neurosci 2017; 20:674-80.
30. Tan TW, Huang YL, Chang JT, Lin JJ, Fong YC, Kuo CC, et al. CCN3 increases BMP-4 expression and bone mineralization in osteoblasts. J Cell Physiol 2012; 227:2531-41.
31. Hui SP, Sheng DZ, Sugimoto K, Gonzalez-Rajal A, Nakagawa S, Hesselson D, et al. Zebrafish Regulatory T Cells Mediate Organ-Specific Regenerative Programs. Dev Cell 2017; 43:659-72 e5.
32. Zaiss DM, van Loosdregt J, Gorlani A, Bekker CP, Grone A, Sibilia M, et al. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. Immunity 2013; 38:275-84.
33. Meulenbroeks C, van Weelden H, Schwartz C, Voehringer D, Redegeld FAM, Rutten V, et al. Basophil-derived amphiregulin is essential for UVB irradiation-induced immune suppression. J Invest Dermatol 2015; 135:222-8.
34. Dai K, Huang L, Chen J, Yang L, Gong Z. Amphiregulin promotes the immunosuppressive activity of intrahepatic CD4(+) regulatory T cells to impair CD8(+) T-cell immunity against hepatitis B virus infection. Immunology 2015; 144:506-17.
35. Dai K, Huang L, Sun X, Yang L, Gong Z. Hepatic CD206-positive macrophages express amphiregulin to promote the immunosuppressive activity of regulatory T cells in HBV infection. J Leukoc Biol 2015; 98:1071-80.
36. Wang S, Zhang Y, Wang Y, Ye P, Li J, Li H, et al. Amphiregulin Confers Regulatory T Cell Suppressive Function and Tumor Invasion via the EGFR/GSK-3beta/Foxp3 Axis. J Biol Chem 2016; 291:21085-95.
37. Minutti C, Modak R, Macdonald F, Li F, Smyth D, Dorward D, et al. A macrophage-pericyte axis directs tissue restoration via Amphiregulin-induced TGFβ activation. Immunity in press.
38. Schneider MR, Werner S, Paus R, Wolf E. Beyond wavy hairs: the epidermal growth factor receptor and its ligands in skin biology and pathology. Am J Pathol 2008; 173:14-24.
39. Linehan JL, Harrison OJ, Han SJ, Byrd AL, Vujkovic-Cvijin I, Villarino AV, et al. Non-classical Immunity Controls Microbiota Impact on Skin Immunity and Tissue Repair. Cell 2018; 172:784-96 e18.
40. Shankaran H, Ippolito DL, Chrisler WB, Resat H, Bollinger N, Opresko LK, et al. Rapid and sustained nuclear-cytoplasmic ERK oscillations induced by epidermal growth factor. Mol Syst Biol 2009; 5:332.
41. Albeck JG, Mills GB, Brugge JS. Frequency-modulated pulses of ERK activity transmit quantitative proliferation signals. Mol Cell 2013; 49:249-61.
42. Gilmore JL, Scott JA, Bouizar Z, Robling A, Pitfield SE, Riese DJ, 2nd, et al. Amphiregulin-EGFR signaling regulates PTHrP gene expression in breast cancer cells. Breast Cancer Res Treat 2008; 110:493-505.

43. Minutti CM, Drube S, Blair N, Schwartz C, McCrae JC, McKenzie AN, et al. Epidermal Growth Factor Receptor Expression Licenses Type-2 Helper T Cells to Function in a T Cell Receptor-Independent Fashion. Immunity 2017; 47:710-22 e6.
44. Gleizes PE, Munger JS, Nunes I, Harpel JG, Mazzieri R, Noguera I, et al. TGF-beta latency: biological significance and mechanisms of activation. Stem Cells 1997; 15:190-7.
45. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 1999; 96:319-28.
46. Burbach BJ, Medeiros RB, Mueller KL, Shimizu Y. T-cell receptor signaling to integrins. Immunol Rev 2007; 218:65-81.
47. Alon R, Shulman Z. Chemokine triggered integrin activation and actin remodeling events guiding lymphocyte migration across vascular barriers. Exp Cell Res 2011; 317:632-41.
48. Lefort CT, Ley K. Neutrophil arrest by LFA-1 activation. Front Immunol 2012; 3:157.
49. Heredia JE, Mukundan L, Chen FM, Mueller AA, Deo RC, Locksley RM, et al. Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. Cell 2013; 153:376-88.
50. Schiering C, Krausgruber T, Chomka A, Frohlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature 2014; 513:564-8.
51. Siede J, Frohlich A, Datsi A, Hegazy AN, Varga DV, Holecska V, et al. IL-33 Receptor-Expressing Regulatory T Cells Are Highly Activated, Th2 Biased and Suppress CD4 T Cell Proliferation through IL-10 and TGFbeta Release. PLoS One 2016; 11:e0161507.
52. Kuswanto W, Burzyn D, Panduro M, Wang KK, Jang YC, Wagers AJ, et al. Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent Accumulation of Regulatory T Cells. Immunity 2016; 44:355-67.
53. Popovic B, Golemac M, Podlech J, Zeleznjak J, Bilic-Zulle L, Lukic ML, et al. IL-33/ST2 pathway drives regulatory T cell dependent suppression of liver damage upon cytomegalovirus infection. PLoS Pathog 2017; 13:e1006345.
54. Liao W, Schones DE, Oh J, Cui Y, Cui K, Roh TY, et al. Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor alpha-chain expression. Nat Immunol 2008; 9:1288-96.
55. Tuettenberg A, Hahn SA, Mazur J, Gerhold-Ay A, Scholma J, Marg I, et al. Kinome Profiling of Regulatory T Cells: A Closer Look into a Complex Intracellular Network. PLoS One 2016; 11:e0149193.
56. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res 2002; 12:9-18.
57. Sprouse ML, Shevchenko I, Scavuzzo MA, Joseph F, Lee T, Blum S, et al. Cutting Edge: Low-Affinity TCRs Support Regulatory T Cell Function in Autoimmunity. J Immunol 2018; 200:909-14.

Figure 1:

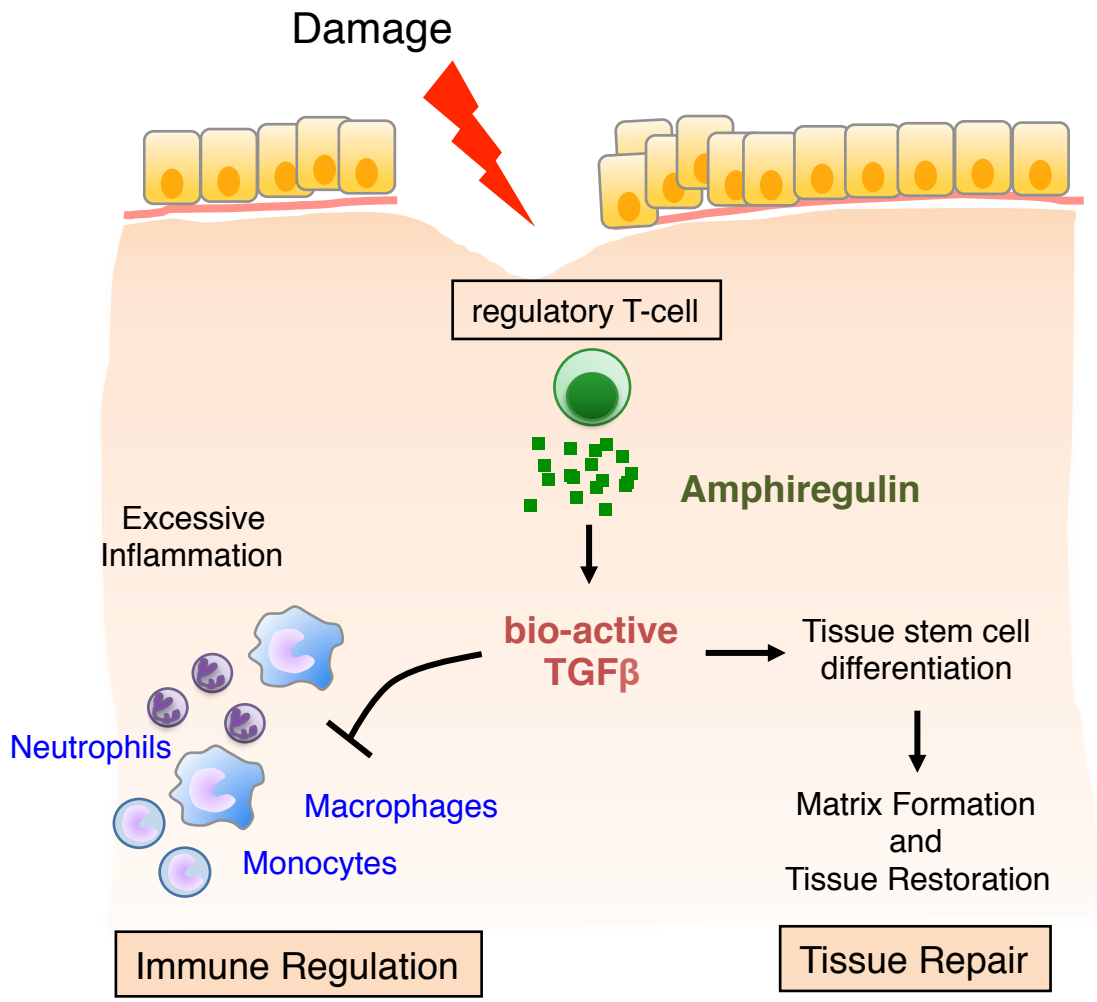

Figure 1: Dual function of regulatory T-cells during wound healing.

The process of wound healing has to be well orchestrated and disruption of this process, for instance due to the infection of the healing wound, can lead to a failure of wound healing or excessive scar formation. Regulatory T-cells rapidly accumulate at sites of injury and keep local immune responses under control so that no excessive immune responses develop that could cause additional damage. This immune regulatory, “damage-limiting” function of regulatory T-cells is complemented by an immune independent, “damage-resolving” direct role in wound healing. In this process, regulatory T-cells at the site of injury release growth factors, such as the EGF-like growth factor Amphiregulin, that directly contribute to the proliferation and differentiation of cells within the injured tissues; in this way, contributing to wound healing and the restoration of tissue homeostasis.

Figure 2:

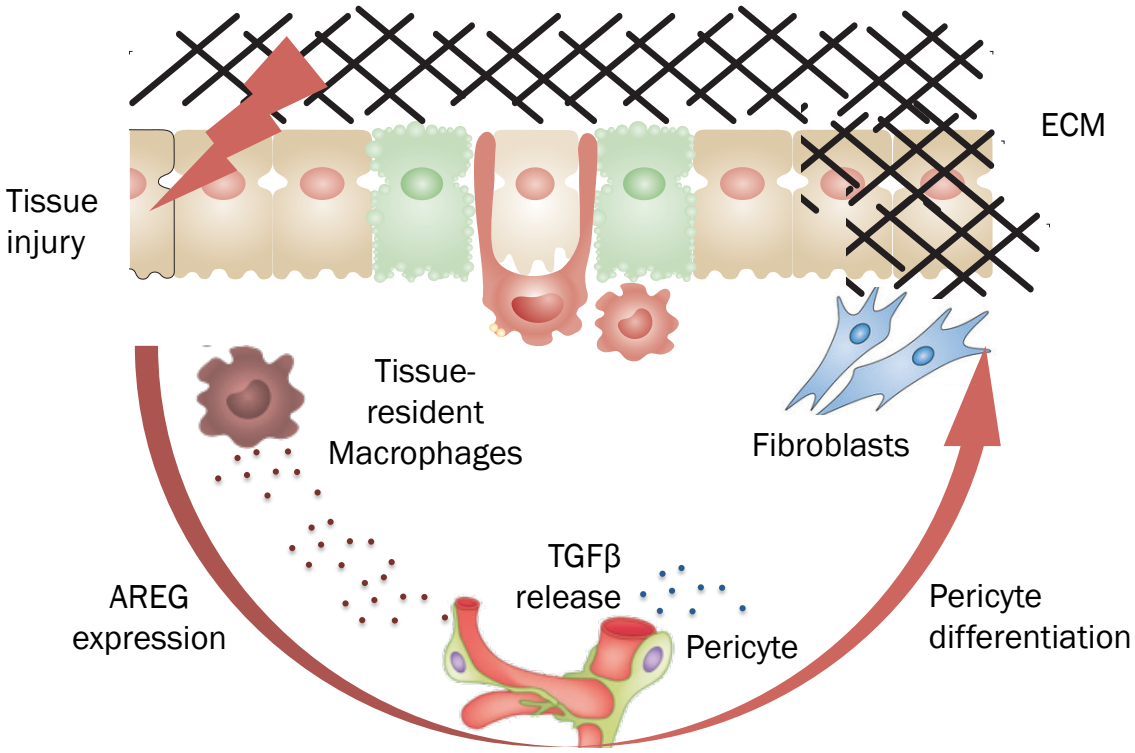

Figure 2: Amphiregulin contributes to wound healing by releasing local, bioactive TGFβ

Upon tissue damage, tissue-resident macrophages sense the breach of tissue homeostasis and release Amphiregulin, which activates the local release of bio-active TGFβ. This local release of bio-active TGFβ induces the differentiation of local pericyte populations into myofibroblasts, which produce extra-cellular matrix components essential for the restoration of blood barrier function and the restoration of tissue homeostasis.

Figure 3:

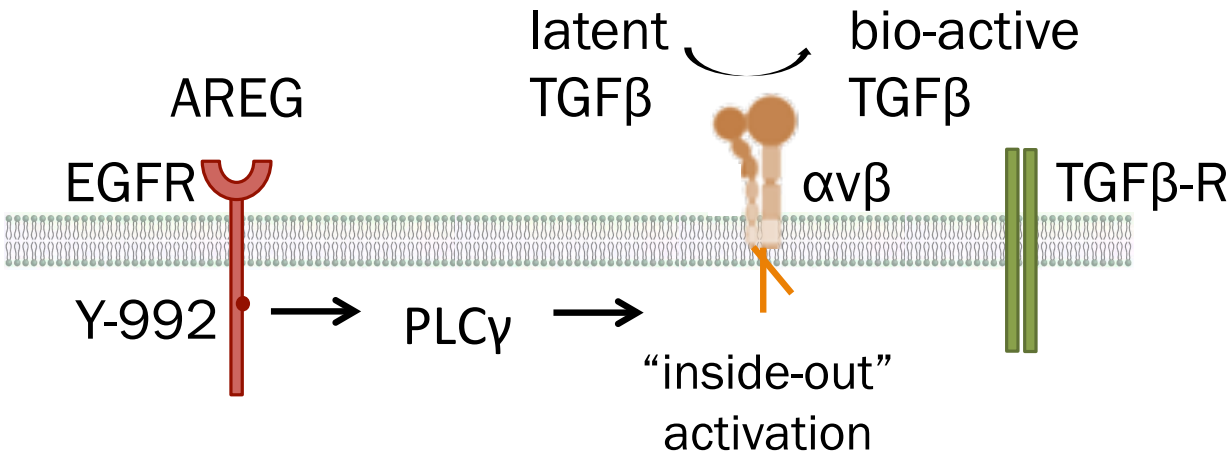

Figure 3: Amphiregulin induces an “inside-out” activation of integrin complexes that then lead to the local release of bioactive TGFβ.

Amphiregulin induces a sustained PLCγ-mediated signal via the Epidermal Growth Factor Receptor (EGFR). Such a sustained PLCγ-mediated signalling induces an intra-cellular rearrangement of local actin fibers and the physical separation of complexed, trans-membrane integrin-α and integrin-β subunits and thus to their extra-cellular activation. Such an “inside-out” activation of integrin-α_V containing complexes can induce the local release of bio-active TGFβ from its latent form, and thus to the local activation of the TGFβ signalling pathway.

Figure 4:

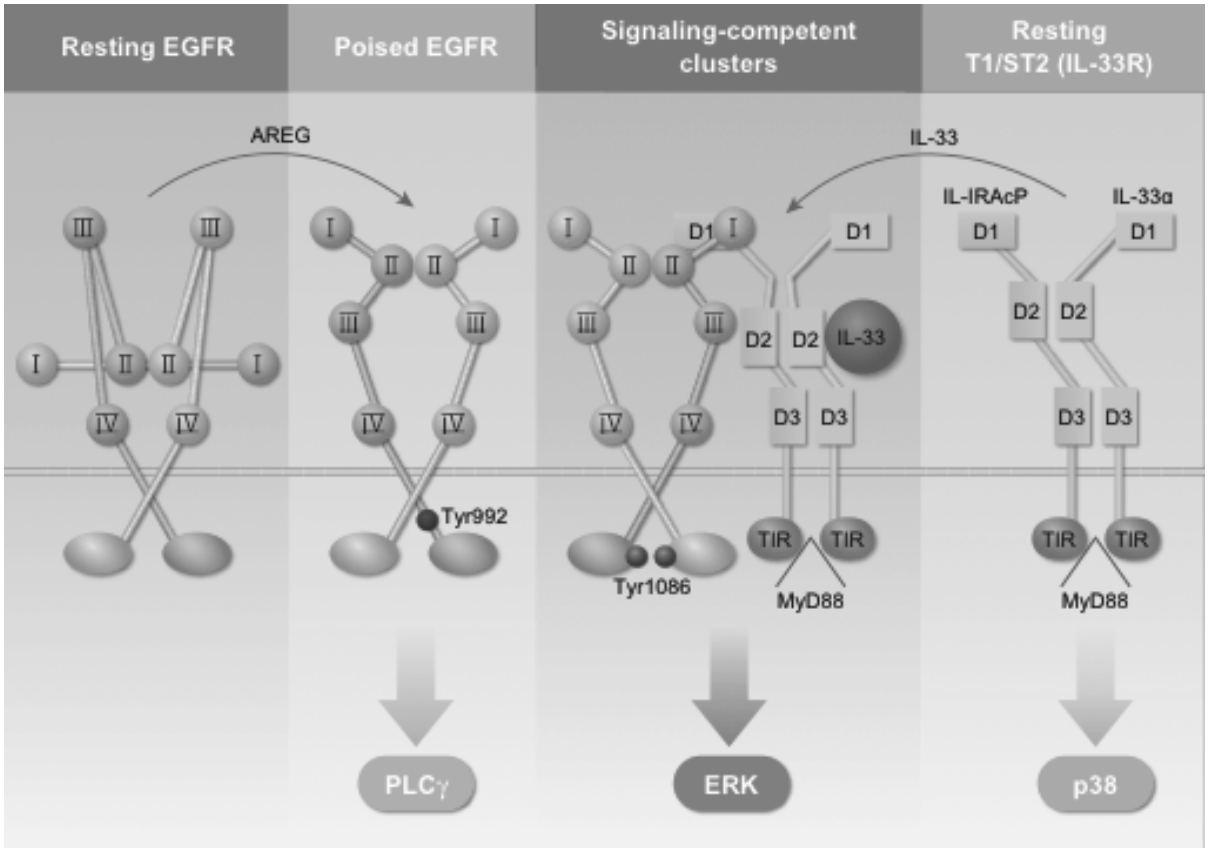

Figure 4: An Amphiregulin-mediated autocrine activation of the EGFR facilitates T-cells to form a hetero-complex with the IL-33R (T1/ST2), which enables IL-33 to activate the MAP-kinase signalling pathway.

The low-affinity EGFR ligand Amphiregulin activates resting EGFR complexes. This places the receptor into a “poised state”, which allows it to enter “signalling competent” clusters on the cell surface and to interact with the IL-33R (T1/ST2). Such clusters of hetero-complexes between the EGFR and T1/ST2 then enables IL-33 to mediate a MAP-kinase mediated signal via the EGFR, and thus potentially the proliferation of tissue-resident regulatory T-cell populations.